Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1-7 and 18-20 are pending in the application, with claim 1 being the sole independent claim. Claims 8-17 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Claims 1 and 18-20 are sought to be amended. These amendments more clearly describe and point out that which Applicants regard as their invention and place the application in condition for allowance. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Rejections Under 35 U.S.C. § 112

Claims 1-20 were finally rejected under 35 U.S.C. § 112, first paragraph, as "containing subject mater which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is mostly nearly connected, to make and/or use the invention." (Office Action, page 2, ¶ 4).

Applicants thank the Examiner for the helpful telephone interview between Applicants' undersigned representative, Steven R. Ludwig, Esq., Examiner Chernyshev

and Primary Examiner John Ulm on February 5, 2003. During the interview, the Examiners stated that in view of claim amendments and cancellation of certain claims, there are two outstanding issues, which are: (a) enablement of the claimed method *in vivo*, and (b) enablement for determining an effective dosage for practicing the claimed method.

With regard to the enablement of the claimed method *in vivo*, the Examiner's attention is drawn to the experiment described in Example 1 (*See* Spec. at ¶ [0090]) utilizing an *in vivo* model of cerebral ischemia. The model mimics glutamate induced N-methyl-D-Aspartate (NMDA) receptor agonism that follows a cerebral ischemic event. *See* Spec. at ¶ [0002]. Such receptor activation leads to depolarization of neuronal membranes and eventually neuronal death. *Id.* Applicants have demonstrated that according to the invention, transient K^+ current is involved in ameliorating neuronal damage caused by depolarization. Compounds such as angiotensin-II antagonists saralasin and losartan, through their antagonism of the angiotensin-II receptor, increase transient K^+ current in excitable cells.

In Example 1, one group of rats received angiotensin-II antagonist (saralasin)/NMDA intracranial injection in the area of the paraventricular nucleus (PVN) of the hypothalamus. Another group of rats received saline/NMDA intracranial injection in the PVN. Three days after injection, the rats were sacrificed and the region of the paraventricular nucleus of the hypothalamus was removed, sectioned, mounted and stained. These histological sections were studied to determine the number of viable neurons. The results depicted in Figure 4 show that rats receiving saralasin during or

after an NMDA-induced ischemic event had significantly more viable neurons than those receiving NMDA. Thus, these *in vivo* data enable the claims of the present invention.

An additional *in vivo* experiment conducted by the present inventors using losartan demonstrates the efficacy of the claimed method in systemic treatment. At page 27, ¶ [0081], the specification reads: "In a preferred embodiment of this invention, the angiotensin-II receptor antagonist is losartan. Losartan has been found to cross the blood-brain barrier []." The experiment involved administering to Male Spontaneously Hypertensive Rats a bolus systemic injection of losartan (30 mg/kg). This was accompanied by an intracranial injection of NMDA receptor agonist *d,l*-(tetrazol-5-yl)glycine) to mimic the glutamate excititoxicity subsequent to an ischemic event. After three days, the rats were sacrificed and the viable neurons were counted as described above. The data show no difference in the numbers of viable neurons between the losartan group and control.

Thus, *in vivo* studies show the ability of local and systemic administration of angiotensin-II antagonists to effect an increase in transient K^+ current, providing a method of preventing damage to the excitable cells that express a transient K^+ current in an animal who is undergoing or has undergone an ischemic event.

Regarding the second issue which concerns dosing regimens, administration of angiotensin-II antagonists is known in the art. Applicants respectfully reassert that it would not require undue experimentation for a skilled artisan to determine an effective dosage of such antagonists to prevent damage to the excitable cells of a patient that express a transient K^+ current. *See* Exhibit A attached hereto (referring to excerpts from U.S. patents cited in the present specification). Clinical data are also enabling in this

regard by showing that the usual dose range of losartan (COZAAR®) in humans for an antihypertensive effect is 25 –100 mg/day. *Physicians' Desk Reference 53rd Ed.*, (1999) p. 1762 (Exhibit B, attached hereto). The lethal dosage of losartan in rats is 2000 mg/kg, which is 170 times the maximum recommended human dose on a mg/m^2 basis. *Id.*

The state of the art in combination with the working examples provides enablement for a skilled artisan to establish an effective dosing regimen using known methods and published angiotensin-II antagonist clinical pharmacology. This type of experimental determination of dosage is of a typical nature to one of ordinary skill in the art, and therefore cannot be considered undue experimentation.

Applicants respectfully submit that the issues of *in vivo* efficacy of the claimed method, and enablement with regard to effective doses to practice the claimed method have been fully addressed.

Information Disclosure Statement

The Examiner has requested that Applicants submit another copy of each of Documents AC1 and AR2. The requested copies are provided herewith.

Conclusion

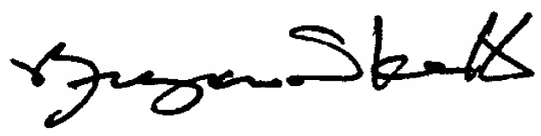
All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be

withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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Version with markings to show changes made

In the Claims:

Claims 8-17 were canceled without prejudice or disclaimer.

Claim 1 was amended as follows:

1. (Twice amended) A method of preventing damage to the excitable cells of a patient that express a transient potassium (K^+) [conductance] current which comprises administering to said patient during or after said patient undergoes or has undergone an ischemic event, an effective amount of [a compound] an angiotensin-II receptor antagonist which increases a transient potassium (K^+) current in said excitable cells of said patient.

Claim 18 was amended as follows:

18. (Once amended) The method of preventing damage to the excitable cells of a patient as claimed in claim [13] 1, wherein said angiotensin-II receptor antagonist crosses the blood-brain barrier.

Claim 19 was amended as follows:

19. (Once amended) The method of preventing damage to the excitable cells of a patient as claimed in claim [13] 1, wherein said angiotensin-II receptor antagonist is losartan.

Claim 20 was amended as follows:

20. (Once amended) The method of preventing damage to the excitable cells of a patient as claimed in claim [13] 1, wherein said angiotensin-II receptor antagonist is saralasin.

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To illustrate these combinations, **one of the angiotensin II antagonists of this invention effective clinically in the 5-500 milligrams per day range** can be effectively combined at levels at the 1.0-500 milligrams per day range with the following compounds at the indicated per day dose range: hydrochlorothiazide (6-100 mg) chlorothiazide (125-500 mg), ethacrynic acid (5-200 mg), amiloride (5-20 mg), furosemide (5-80 mg), propranolol (10-480 mg), timolol maleate (1-20 mg.), methyldopa (125-2000 mg), felodipine (1-20 mg), nifedipine (5-120 mg), nitrendipine (5-60 mg) and diltizaem (30-540 mg). In addition, triple drug combinations of hydrochlorothiazide (5-100 mg) plus amiloride (5-20 mg) plus angiotensin II antagonist of this invention (1-500 mg) or hydrochlorothiazide (5-100 mg) plus timolol maleate (5-60) plus an angiotensin II antagonist of this invention (1-500 mg) or hydrochlorothiazide (5-200 mg) and nifedipine (5-60 mg) plus an angiotensin II antagonist of this invention (1-500 mg) are effective combinations to control blood pressure in hypertensive patients. Naturally, these dose ranges can be adjusted on a unit basis as necessary to permit divided daily dosage and, as noted above, the dose will vary depending on the nature and severity of the disease, weight of patient, special diets and other factors.

About 1 to 100 mg. of compound or mixture of compounds of Formula I or a physiologically acceptable salt is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is such that a suitable dosage in the range indicated is obtained.

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To illustrate these combinations, one of the angiotensin II antagonists of this invention effective clinically in the 2.5-250 milligrams per day range can be effectively combined at levels at the 0.5-250 milligrams per day range with the following compounds at the indicated per day dose range: hydrochlorothiazide (15-200 mg) chlorothiazide (125-2000 mg), ethacrynic acid (15-200 mg), amiloride (5-20 mg), furosemide (5-80 mg), propranolol (20-480 mg), timolol maleate (5-60 mg.), methyldopa (65-2000 mg), felodipine (5-60 mg), nifedipine (5-60 mg), and nitrendipine (5-60 mg). In addition, triple drug combinations of hydrochlorothiazide (15-200 mg) plus amiloride (5-20 mg) plus angiotensin II antagonist of this invention (3-200 mg) or hydrochlorothiazide (15-200 mg) plus timolol maleate (5-60) plus an angiotensin II antagonist of this invention (0.5-250 mg) or hydrochlorothiazide (15-200 mg) and nifedipine (5-60 mg) plus an angiotensin II antagonist of this invention (0.5-250 mg) are effective combinations to control blood pressure in hypertensive patients. Naturally, these dose ranges can be adjusted on a unit basis as necessary to permit divided daily dosage and, as noted above, the dose will vary depending on the nature and severity of the disease, weight of patient, special diets and other factors.

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To illustrate these combinations, one of the angiotensin II antagonists of this invention effective clinically in the 2.5-250 milligrams per day range can be effectively combined at levels at the 0.5-250 milligrams per day range with the following compounds at the indicated per day dose range: hydrochlorothiazide (15-200 mg), chlorothiazide (125-2000 mg), ethacrynic acid (15-200 mg), amiloride (5-20 mg), furosemide (5-80 mg), propranolol (20-480 mg), timolol maleate (5-60 mg), methyldopa (65-2000 mg), felodipine (5-60 mg), nifedipine (5-60 mg), and nitrendipine (5-60 mg). In addition, triple drug combinations of hydrochlorothiazide (15-200 mg) plus amiloride (5-20 mg) plus angiotensin II antagonist of this invention (3-200 mg) or hydrochlorothiazide (15-200 mg) plus timolol maleate (5-60) plus an angiotensin II antagonist of this invention (0.5-250 mg) or hydrochlorothiazide (15-200 mg) and nifedipine (5-60 mg) plus an angiotensin II antagonist of this invention (0.5-250 mg) are effective combinations to control blood pressure in

hypertensive patients. Naturally, these dose ranges can be adjusted on a unit basis as necessary to permit divided daily dosage and, as noted above, the dose will vary depending on the nature and severity of the disease, weight of patient, special diets and other factors.

Cozaar—Cont.

ADVERSE REACTIONS

COZAAR has been evaluated for safety in more than 3300 patients treated for essential hypertension and 4058 patients/subjects overall. Over 1200 patients were treated for over 6 months and more than 800 for over one year. In general, treatment with COZAAR was well-tolerated. The overall incidence of adverse experiences reported with COZAAR was similar to placebo.

In controlled clinical trials, discontinuation of therapy due to clinical adverse experiences was required in 2.3 percent of patients treated with COZAAR and 3.7 percent of patients given placebo.

The following table of adverse events is based on four 6–12 week placebo controlled trials involving over 1000 patients on various doses (10–150 mg) of losartan and over 300 patients given placebo. All doses of losartan are grouped because none of the adverse events appeared to have a dose-related frequency. The table includes all adverse events, whether or not attributed to the treatment, occurring in at least 1% of patients treated with losartan and that were more frequent on losartan than placebo.

	Losartan (n=1075) Incidence	Placebo (n=334) Incidence
Digestive		
Diarrhea	2.4	2.1
Dyspepsia	1.3	1.2
Musculoskeletal		
Cramp, muscle	1.1	0.3
Myalgia	1.0	0.9
Pain, back	1.8	1.2
Pain, leg	1.0	0.0
Nervous System / Psychiatric		
Dizziness	3.5	2.1
Insomnia	1.4	0.6
Respiratory		
Congestion, nasal	2.0	1.2
Cough	3.4	3.3
Infection, upper respiratory	7.9	6.9
Sinus disorder	1.5	1.2
Sinusitis	1.0	0.3

The following adverse events were also reported at a rate of 1% or greater in patients treated with losartan, but were as, or more frequent, in the placebo group: asthenia/fatigue, edema/swelling, abdominal pain, chest pain, nausea, headache, pharyngitis.

Adverse events occurred at about the same rates in men and women, older and younger patients, and black and non-black patients.

A patient with known hypersensitivity to aspirin and penicillin, when treated with COZAAR, was withdrawn from study due to swelling of the lips and eyelids and facial rash, reported as angioedema, which returned to normal 5 days after therapy was discontinued.

Superficial peeling of palms and hemolysis was reported in one subject.

In addition to the adverse events above, potentially important events that occurred in at least two patients/subjects exposed to losartan or other adverse events that occurred in <1% of patients in clinical studies are listed below. It cannot be determined whether these events were causally related to losartan: *Body as a Whole:* facial edema, fever, orthostatic effects, syncope; *Cardiovascular:* angina pectoris, second degree AV block, CVA, hypotension, myocardial infarction, arrhythmias including atrial fibrillation, palpitation, sinus bradycardia, tachycardia, ventricular tachycardia, ventricular fibrillation; *Digestive:* anorexia, constipation, dental pain, dry mouth, flatulence, gastritis, vomiting; *Hematologic:* anemia; *Metabolic:* gout; *Musculoskeletal:* arm pain, hip pain, joint swelling, knee pain, musculoskeletal pain, shoulder pain, stiffness, arthralgia, arthritis, fibromyalgia, muscle weakness; *Nervous System / Psychiatric:* anxiety, anxiety disorder, ataxia, confusion, depression, dream abnormality, hypesthesia, decreased libido, memory impairment, migraine, nervousness, paresthesia, peripheral neuropathy, panic disorder, sleep disorder, somnolence, tremor, vertigo; *Respiratory:* dyspnea, bronchitis, pharyngeal discomfort, epistaxis, rhinitis, respiratory congestion; *Skin:* alopecia, dermatitis, dry skin, ecchymosis, erythema, flushing, photosensitivity, pruritus, rash, sweating, urticaria; *Special Senses:* blurred vision, burning/stinging in the eye, conjunctivitis, taste perversion, tinnitus, decrease in visual acuity; *Urogenital:* impotence, nocturia, urinary frequency, urinary tract infection.

Post-Marketing Experience

The following additional adverse reactions have been reported in post-marketing experience: *Hypersensitivity:* Angioedema (involving swelling of the face, lips, pharynx, and/or tongue) has been reported rarely in patients treated with losartan; some of these patients previously experienced angioedema with other drugs including ACE inhibitors; *Digestive:* Hepatitis (reported rarely).

Hyperkalemia has been reported.

Laboratory Test Findings

In controlled clinical trials, clinically important changes in standard laboratory parameters were rarely associated with administration of COZAAR.

Creatinine, Blood Urea Nitrogen: Minor increases in blood urea nitrogen (BUN) or serum creatinine were observed in less than 0.1 percent of patients with essential hypertension treated with COZAAR alone.

Hemoglobin and Hematocrit: Small decreases in hemoglobin and hematocrit (mean decreases of approximately 0.11 grams percent and 0.09 volume percent, respectively) occurred frequently in patients treated with COZAAR alone, but were rarely of clinical importance. No patients were discontinued due to anemia.

Liver Function Tests: Occasional elevations of liver enzymes and/or serum bilirubin have occurred. In patients with essential hypertension treated with COZAAR alone, one patient (<0.1%) was discontinued due to these laboratory adverse experiences.

OVERDOSAGE

Significant lethality was observed in mice and rats after oral administration of 1000 mg/kg and 2000 mg/kg, respectively, about 44 and 170 times the maximum recommended human dose on a mg/m^2 basis.

Limited data are available in regard to overdosage in humans. The most likely manifestation of overdosage would be hypotension and tachycardia; bradycardia could occur from parasympathetic (vagal) stimulation. If symptomatic hypotension should occur, supportive treatment should be instituted.

Neither losartan nor its active metabolite can be removed by hemodialysis.

DOSAGE AND ADMINISTRATION

The usual starting dose of COZAAR is 50 mg once daily, with 25 mg used in patients with possible depletion of intravascular volume (e.g., patients treated with diuretics) (see WARNINGS, *Hypotension—Volume-Depleted Patients*) and patients with a history of hepatic impairment (see PRECAUTIONS, *General*). COZAAR can be administered once or twice daily with total daily doses ranging from 25 mg to 100 mg.

If the antihypertensive effect measured at trough using once-a-day dosing is inadequate, a twice-a-day regimen at the same total daily dose or an increase in dose may give a more satisfactory response.

If blood pressure is not controlled by COZAAR alone, a low dose of a diuretic may be added. Hydrochlorothiazide has been shown to have an additive effect (see CLINICAL PHARMACOLOGY, *Pharmacodynamics and Clinical Effects*).

No initial dosage adjustment is necessary for elderly patients or for patients with renal impairment, including patients on dialysis.

COZAAR may be administered with other antihypertensive agents.

COZAAR may be administered with or without food.

HOW SUPPLIED

No. 3612—Tablets COZAAR, 25 mg, are light green, teardrop-shaped, film-coated tablets with code MRK on one side and 951 on the other. They are supplied as follows:

NDC 0006-0951-54 unit of use bottles of 90
(6505-01-414-4064, 25 mg 90's)
NDC 0006-0951-58 unit of use bottles of 100
(6505-01-414-4059, 25 mg 100's)
NDC 0006-0951-28 unit dose packages of 100
(6505-01-414-4063, 25 mg individually sealed 100's).

Shown in Product Identification Guide, page 323

No. 3613—Tablets COZAAR, 50 mg, are green, teardrop-shaped, film-coated tablets with code MRK 952 on one side and COZAAR on the other. They are supplied as follows:

NDC 0006-0952-31 unit of use bottles of 30
(6505-01-414-4062, 50 mg 30's)
NDC 0006-0952-54 unit of use bottles of 90
(6505-01-414-4060, 50 mg 90's)
NDC 0006-0952-58 unit of use bottles of 100
(6505-01-414-4058, 50 mg 100's)
NDC 0006-0952-28 unit dose packages of 100
(6505-01-414-4061, 50 mg individually sealed 100's)
NDC 0006-0952-82 bottles of 1,000.

Shown in Product Identification Guide, page 323

Storage

Store at controlled room temperature, 15–30°C (59–86°F).
Keep container tightly closed. Protect from light.

Manufactured for:
MERCK & CO., INC., West Point, PA 19486, USA
by:
Du Pont Pharmaceuticals, Wilmington, DE 19880 USA

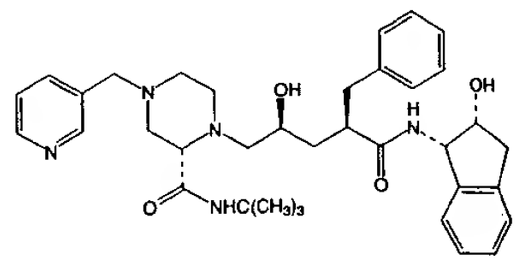
7882907 Issued March 1998

CRIXIVAN® Capsules
(indinavir sulfate), U.S.P.

DESCRIPTION

CRIXIVAN* (indinavir sulfate) is an inhibitor of the human immunodeficiency virus (HIV) protease. CRIXIVAN Capsules are formulated as a sulfate salt and are available for oral administration in strengths of 200 and 400 mg of indinavir (corresponding to 250 and 500 mg indinavir sulfate, respectively). Each capsule also contains the inactive ingredients anhydrous lactose and magnesium stearate. The capsule shell has the following inactive ingredients and dyes: gelatin, titanium dioxide, silicon dioxide and sodium lauryl sulfate.

The chemical name for indinavir sulfate is [1(1*S*,2*R*),5(*S*)-2,3,5-trideoxy-*N*-(2,3-dihydro-2-hydroxy-1 *H*-inden-1-yl)-5-[2-[[(1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl] -2 -(phenylmethyl)-D-*erythro*-pentonamide sulfate (1:1) salt. Indinavir sulfate has the following structural formula:



Indinavir sulfate is a white to off-white, hygroscopic, crystalline powder with the molecular formula $C_{36}H_{47}N_5O_4 \bullet H_2SO_4$ and a molecular weight of 711.88. It is very soluble in water and in methanol.

*Registered trademark of MERCK & CO., Inc.

MICROBIOLOGY

Mechanism of Action: HIV protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV. Indinavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature noninfectious viral particles.

Antiretroviral Activity In Vitro: The relationship between *in vitro* susceptibility of HIV to indinavir and inhibition of HIV replication in humans has not been established. The *in vitro* activity of indinavir was assessed in cell lines of lymphoblastic and monocytic origin and in peripheral blood lymphocytes. HIV variants used to infect the different cell types include laboratory-adapted variants, primary clinical isolates and clinical isolates resistant to nucleoside analogue and nonnucleoside inhibitors of the HIV reverse transcriptase. The IC_{95} (95% inhibitory concentration) of indinavir in these test systems was in the range of 25 to 100 nM. In drug combination studies with the nucleoside analogues zidovudine and didanosine, as well as with an investigational nonnucleoside (L-697,661), indinavir showed synergistic activity in cell culture.

Drug Resistance: Isolates of HIV with reduced susceptibility to the drug have been recovered from some patients treated with indinavir. Viral resistance was correlated with the accumulation of mutations that resulted in the expression of amino acid substitutions in the viral protease. Eleven amino acid residue positions, at which substitutions are associated with resistance, have been identified. Resistance was mediated by the co-expression of multiple primary variable substitutions at these positions. In general, higher levels of resistance were associated with the co-expression of greater numbers of substitutions.

Cross-Resistance to Other Antiviral Agents: Cross-resistance was noted between indinavir and the protease inhibitor ritonavir. Varying degrees of cross-resistance have been observed between indinavir and other HIV-protease inhibitors.

CLINICAL PHARMACOLOGY

Pharmacokinetics

Absorption: Indinavir was rapidly absorbed in the fasted state with a time to peak plasma concentration (T_{max}) of 0.8 ± 0.3 hours (mean ± S.D.) (n=11). A greater than dose-proportional increase in indinavir plasma concentration was observed over the 200–1000 mg dose range. At a dose